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NOTES

Fungicidal Monoclonal Antibody C7 Binds to *Candida albicans* Als3▽

Sonia Brena,[1] Miren J. Omaetxebarría,[1] Natalia Elguezabal,[1] Jonathan Cabezas,[1] María D. Moragues,[2] and José Pontón[1]*

Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología,[1] and Departamento de Enfermería I,[2] Universidad del País Vasco, Bilbao, Vizcaya, Spain

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Monoclonal antibody (MAb) C7 reacted with a >200-kDa component from the *Candida albicans* cell wall identified by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry as Als3. It also bound the recombinant N terminus of Als3. Binding of MAb C7 to Als3 may explain the biological activities exerted by the MAb on *C. albicans*.

Monoclonal antibody (MAb) C7 was raised against a protein epitope of a stress mannoprotein of >200 kDa that is the main target of salivary secretory immunoglobulin A (IgA) in the cell wall of *Candida albicans* (7). This MAb exhibits potent fungicidal activity against *C. albicans*, *Candida lusitaniae*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Scedosporium prolificans* (5), and it has been shown to protect mice from invasive candidiasis (10). In addition to the fungicidal activity, MAb C7 also exerts other biological activities, including inhibition of germination of *C. albicans*, inhibition of adhesion of *C. albicans* to buccal epithelial cells (BECs), and direct tumoricidal activity (5, 6). Identification of the antigen recognized by MAb C7 may lead to understanding of its mechanism of action and to development of new antifungal and antitumor drugs. In this report, we identify the antigen recognized by MAb C7 on the cell wall surface of *C. albicans*.

Candida albicans NCPF 3153 from the National Collection of Pathogenic Fungi, Bristol, United Kingdom, was used in this study. Blastospores and germ tubes were grown in medium 199 (Sigma-Aldrich, Steinheim, Germany) as described previously (5). *Escherichia coli*(DE3)(pLacI) (Novagen, Darmstadt, Germany) was used as the host strain for the recombinant plasmid (pTriEx1 vector; Novagen). *E. coli* cells were grown in Luria-Bertani (LB) medium or on LB agar plates supplemented, when necessary, with carbenicillin (50 μg/ml) and chloramphenicol (34 μg/ml) (Sigma).

The cell wall of *C. albicans* was extracted in the presence of dithiothreitol (Sigma) as reported previously (8). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting were performed as reported previously (5). The *C. albicans* germ tube stress mannoprotein of >200 kDa recognized by MAb C7 was separated in 7.5% acrylamide gels and electroeluted as described previously (5). Fractions of the mannoprotein of >200 kDa were selected on the basis of their reactivity against MAb C7 and were collected and concentrated using Microcon YM-10 at 13,000 rpm for 15 min (Millipore Ibérica, Madrid, Spain). The antigen was deglycosylated with *N*-glycosidase F, using a glycoprotein deglycosylation kit (Calbiochem, Darmstadt, Germany). In some experiments, removal of carbohydrate groups from the antigen was accomplished by oxidation with sodium *meta*-periodate (5). Deglycosylated samples were analyzed by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry.

Primers 5′-CGGGATCCGGCAAAGACAATCACTGGT GTTTTC-3′ and 5′-GGGGTACACATATAGAATCTTCCA AATC-3′ were used to clone the N-terminal fragment of *ALS3* by standard techniques (9). A recombinant fragment of *C. albicans HWP1* cloned in the same plasmid employed to clone the N-terminal fragment of *ALS3* was used as control (3).

MAb C7 has been previously described (5). An irrelevant mouse IgM MAb was used as control. Polyclonal antisera against two peptides designed on the basis of a specific sequence located on the C-terminal region of *C. albicans* Als3 (CSWVSVSTRI, anti-Als3) and a conserved sequence of all *C. albicans* Als proteins (NPTVTTTEYW, anti-Als) were obtained in New Zealand White rabbits by Sigma-Genosys (Suffolk, United Kingdom). Preimmune serum did not react with the deglycosylated >200-kDa antigen.

The inhibition of germination of *C. albicans*, the inhibition of adhesion of *C. albicans* to BECs, and the candidacidal activity mediated by MAb C7 and the anti-Als3 and anti-Als antisera were studied as described previously (5).

Deglycosylation of the >200-kDa stress mannoprotein eluted from dithiothreitol extracts from *C. albicans* germ tubes yielded a band of lower molecular mass (Fig. 1, lanes 1 and 2) which was recognized by MAb C7 after further oxidation of the antigen with sodium *meta*-periodate (Fig. 1, lanes 3 and 4). The need for the oxidation with sodium *meta*-periodate suggested that deglycosylation with the glycoprotein deglycosylation kit was not complete. The deglycosylated component was identified by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry as the agglutinin-like cell surface pro-

* Corresponding author. Mailing address: Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Apartado 699, E-48080 Bilbao, Vizcaya, Spain. Phone: 34 94-601-2855. Fax: 34 94-601-3495. E-mail: jose.ponton@ehu.es.

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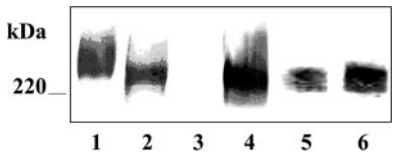

FIG. 1. Western blots of 10% slab gels loaded with an antigen of >200 kDa purified by electroelution from extracts of *C. albicans* germ tubes before (lane 1) and after (lanes 2 to 6) deglycosylation. Antigens were stained with silver stain (lanes 1 and 2), MAb C7 (lanes 3 and 4), a polyclonal antibody against Als3 protein (lane 5), and a polyclonal antibody against Als proteins (lane 6). Antigens in lane 4 were further oxidized with sodium *meta*-periodate. The molecular mass of a standard protein is listed on the left.

tein 3 of *C. albicans* (Als3). Further evidence confirming the identification of the component of >200 kDa as Als3 was obtained with two rabbit antisera produced against two peptides of Als3. Each antiserum reacted with the peptide used for the immunization of the rabbits (Fig. 2A), and both antisera reacted with the deglycosylated >200-kDa antigen (Fig. 1, lanes 5 and 6).

In an attempt to confirm that MAb C7 reacted with Als3, we obtained an N-terminal fragment of the *C. albicans ALS3* gene. The recombinant protein reacted by immunoblotting with MAb C7 (Fig. 2B, lane 1). As expected, both anti-Als3 and anti-Als antisera did not react with the N-terminal end of recombinant Als3, since it did not contain the sequences recognized by the antisera (Fig. 2B, lanes 2 and 3). The specificity of the reactivity of MAb C7 with the N-terminal fragment of the recombinant Als3 was proven by using an irrelevant IgM MAb (Fig. 2B, lane 4) and a recombinant protein cloned in the same plasmid employed to clone the N-terminal end of *ALS3* (Fig. 2B, lane 5).

The ability of anti-Als3 and anti-Als polyclonal antisera to reproduce some of the biological activities of MAb C7 was also studied. None of the polyclonal antisera exhibited candidacidal activity. However, compared to the controls without antibody, anti-Als3 and anti-Als antisera caused 46.3% and 37.3% inhibition of adhesion of *C. albicans* to BECs, respectively ($P < 0.05$), and 66.6% and 36.7% decreases in the filamentation of *C. albicans*, respectively ($P < 0.01$).

Als3p is a member of the *C. albicans* Als protein family, which has a role in the adhesion and virulence of this fungus (1). Als proteins display the N-terminal end on the outer part of cell wall through a central tandemly repeated domain, while the C-terminal end with a glycosylphosphatidylinositol motif allows anchoring to the cell wall (1, 4). These proteins present a high degree of O glycosylation that could explain the limited degree of deglycosylation obtained by the treatment of the >200-kDa antigen with *N*-glycosidase F (2).

The epitope recognized by MAb C7 is not presently known. We have previously reported that MAb C7 reacts with *C. albicans* enolase and the nuclear pore protein Nup88 (6). However, since there is no linear homology between the sequences of Als3, enolase, and Nup88, the epitope may be discontinuous or conformational.

Binding of MAb C7 to Als3 may explain the inhibition of adhesion of *C. albicans* to BECs and the inhibition of germination mediated by MAb C7, since the anti-Als3 and anti-Als antisera caused an inhibition of adhesion of *C. albicans* to

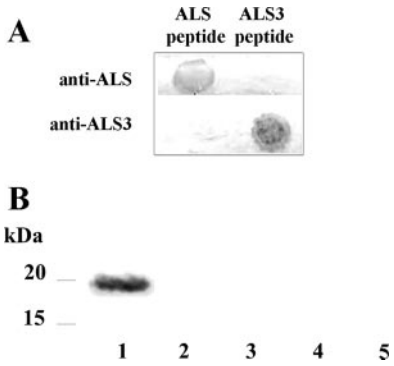

FIG. 2. (A) Dot assay showing the reactivity of polyclonal antisera raised against a peptide with a sequence specific for Als3 and against a peptide with a sequence conserved in all Als proteins. (B) Western blots of 10% slab gels loaded with extracts from *E. coli* cells expressing the N-terminal end of *C. albicans* Als3 stained with MAb C7 (lane 1), a rabbit polyclonal anti-Als3 antiserum (lane 2), a rabbit polyclonal anti-Als antiserum (lane 3), and an irrelevant IgM MAb (lane 4), respectively. A recombinant fragment of *C. albicans HWP1* cloned in the same plasmid used to clone *ALS3* stained with MAb C7 is shown in lane 5. Molecular masses of standard proteins are listed to the left of the gel.

BECs and an inhibition of germination of *C. albicans* similar to that mediated by MAb C7. *ALS1*, *ALS3*, and *ALS5* are at least 85% identical within the 5′ domain (1). Interestingly, a MAb that recognizes the N-terminal end of Als1 caused a 70% inhibition of the epithelial adhesion of *Saccharomyces cerevisiae* expressing *ALS1* (4). Vaccination with Als1 improved survival in murine models of disseminated candidiasis by stimulation of cell-mediated immunity (11). Binding of MAb C7 to the N-terminal end of Als3 may also explain the candidacidal activity of MAb C7, since this activity is neutralized by incubating the MAb with the recombinant N-terminal end of Als3 (data not shown). However, since MAb C7 reacts with different antigens, we cannot exclude that the candidacidal activity of MAb C7 is related to the interaction with an antigen different from Als3.

At present we do not have any evidence for a relationship between Als3 and the tumoricidal activity displayed by MAb C7. Since no evidence of the presence of Als3 or Als proteins in tumor cells has been found, we suspect that MAb C7 reacts with a common epitope present in Als3 and other cell antigens from tumor cells.

In conclusion, MAb C7 binds the Als3 protein of *C. albicans*, an interaction that may explain the inhibition of the adhesion of *C. albicans* to BECs, the inhibition of germination of *C. albicans*, and the candidacidal activity mediated by this antibody.

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